Antibodies active against a fusion polypeptide comprising a histidine portion

The present invention relates to antibodies which are active against a fusion polypeptide comprising a histidine portion, a process for the preparation thereof and their use.

It is known to express a polypeptide in the form of a histidine fusion polypeptide. In such a polypeptide, a histidine portion of e.g. 6-18 successive histidine residues is fused to the C or N terminus of the polypeptide. Hence it is possible to isolate the histidine fusion polypeptide by means of a nickel-chelate chromatographic column from the supernatant or cell lysate of the cell expressing it.

However, the above column is expensive. Furthermore, its use costs a lot of time. Therefore, it is not suited for the rapid detection of the expression of a histidine fusion polypeptide. But such a detection is necessary, particularly when it shall be used for screening many cells.

Thus, it is the object of the present invention to provide means by which the expression of a histidine fusion polypeptide can be detected rapidly.

According to the invention this is achieved by an antibody which is directed against a fusion polypeptide comprising a histidine portion.

Such an antibody may be a polyclonal or monoclonal antibody, a monoclonal antibody being preferred. The antibody may be obtained from any animal or human being, rabbits being preferred for a polyclonal antibody and mice being preferred for a monoclonal antibody.

In addition, the antibody may be synthetic, portions which are not necessary for the above-mentioned identification

optionally lacking fully or partially therefrom and these portions being replaced by others which give the antibody further favorable properties, respectively.

The expression "fusion polypeptide comprising a histidine portion" comprises a polypeptide (peptide) of any kind and length which has a histidine portion. Such a polypeptide may be expressed by any cells, e.g. bacteria, yeasts, cells of insects, plants and animals, as well as organisms, e.g. transgenic animals. An above histidine portion may comprise e.g. 6-18, preferably 6, successive histidine residues and be fused to the N and/or C terminus of the polypeptide.

A preferred antibody of the present invention, namely a monoclonal mouse antibody having the above identification, was deposited under No. ACC 2207 with the DSM [German-type collection of microorganisms] on February 15, 1995.

Antibodies according to the invention can be prepared according to conventional methods. If polyclonal antibodies and monoclonal antibodies, respectively, are to be prepared, it will be favorable to immunize animals, particularly rabbits for the former antibodies and mice for the latter antibodies, with an above histidine fusion polypeptide e.g. His p53 (cf. German patent application P 42 32 823.3) or His hdm2 (cf. German patent application P 43 39 553.3), preferably a mixture thereof. The animals can be further boostered with the same histidine fusion polypeptide or polypeptides. Other histidine fusion polypeptides or a combination of these and the preceding histidine fusion polypeptide or polypeptides may also be used for boostering. The polyclonal antibodies may then be obtained from the serum of the animals. Spleen cells of the animals are fused with myeloma cells for the monoclonal antibodies.

For the preparation of synthetic antibodies, e.g. the above-obtained monoclonal antibodies may be used as a basis. For this purpose, it is the obvious thing to analyze

the antigen-binding region of the monoclonal antibodies and identify the portions which are necessary and not necessary for the above identification. The necessary portions may then be modified and the non-necessary portions can be fully or partially eliminated and replaced by portions giving the antibodies further favorable properties, respectively. Also, portions can be modified, eliminated or replaced beyond the binding regions of the antibodies. A person skilled in the art knows that particularly the DNA recombination technology is suitable for the above measures. He is perfectly familiar therewith.

Antibodies according to the invention distinguish themselves in that they recognize any fusion polypeptides comprising a histidine portion. Therefore, the antibodies are suitable for the rapid detection of the expression of such fusion polypeptides. This may be carried out in any detection methods, particularly in a Western blot, an ELISA, an immunoprecipitation or an immunofluorescence. For this purpose, the antibodies according to the invention may be labeled, if appropriate, or used in combination with labeled antibodies directed thereagainst.

The present invention is explained by the below examples.

Example 1: Preparation of monoclonal antibodies

Mice were used for immunization. His hdm2 (amino acid 1-284), His hdm2 (amino acid 58-491) and His p53 (amino acid 66-393) (cf. above) were used as antigens. They were dissolved in a buffer comprising 8 M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl.

Immunization and booster pattern:

Day 1: 50 µl (= 10 µg) His hdm2 (amino acid 1-284)
50 µl (= 10 µg) His hdm2 (amino acid 58-491)
50 µl PBS (phosphate-buffered saline)
150 µl Freund's adjuvant complete
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300 µl mix

200 µl of the mix were injected into a mouse

Day 30: 50 µl (= 10 µg) His hdm2 (amino acid 1-284)
50 µl (= 10 µg) His hdm2 (amino acid 58-491)
20 µl PBS
120 µl Freund's adjuvant incomplete
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240 µl mix

200 µl of the mix were injected into the above mouse.

Day 60: 50 µl (= 10 µg) His hdm2 (amino acid 1-284)
50 µl (= 10 µg) His hdm2 (amino acid 58-491)
85 µl PBS
115 µl Freund's adjuvant incomplete
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300 µl mix

200 µl of the mix were injected into the above mouse.

Day 90: 50 µl (= 10 µg) His hdm2 (amino acid 1-284)
50 µl (= 10 µg) His hdm2 (amino acid 58-491)
200 µl PBS
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300 µl mix

200 µl of the mix were injected into the above mouse.

Day 180: 150 µl (= 20 µg) His p53 (amino acid 66-393)
150 µl Freund's adjuvant complete
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300 µl mix

200 µl of the mix were injected into the above mouse.

Day 230: 75 µl (= 10 µg) His p53 (amino acid 66-393)
25 µl (= 5 µg) His hdm2 (amino acid 1-284)
25 µl (= 5 µg) His hdm2 (amino acid 58-491)
125 µl Freund's adjuvant incomplete
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250 µl mix

200 µl of the mix were injected into the above mouse.

Day 260: 75 µl (= 10 µg) His p53 (amino acid 66-393)
25 µl (= 5 µg) His hdm2 (amino acid 1-284)
25 µl (= 5 µg) His hdm2 (amino acid 58-491)
125 µl PBS
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250 µl mix

200 ml of the mix were injected into the above mouse.

The mouse was killed on day 262. Spleen cells were removed therefrom and fused with myeloma cells. Monoclonal antibodies were obtained. One of them was deposited under ACC 2207 with DSM on February 15, 1995.

Example 2: Preparation of polyclonal antibodies

Rabbits were used for immunization. The antigens of Example 1 were employed. The immunization and booster pattern was identical with that of Example 1 up to day 90 inclusive.

Day 92: 5 ml of blood were removed from the rabbit's auricular vein and tested for antibody activity in an ELISA and Western blot, respectively.

Day 93: Following a positive test on day 92, the animals were killed and the antibodies were obtained from the serum.

Example 3: Detection of histidine fusion polypeptides by antibodies according to the invention

(a) Western blot

Histidine fusion polypeptides, namely His hdm2 (amino acid 1-284), His hdm2 (amino acid 58-491) and His p53 (amino acid 66-393) of Example 1, as well as the polypeptides hdm2 (amino acid 1-284), WAF 1 (= wild type-activating factor) and t16 (= cell-regulating protein) as control were subjected to a polyacrylamide gel eletrophoresis. The gel was transferred overnight to a nitrocellulose membrane. It was then incubated with the above antibody ACC 2207 diluted in a ratio of 1:10 and 1:50, respectively, at 37°C for 1 hour. After several wash steps using PBS (0.05 % Tween 20), a purchasable alkaline phosphatase-coupled goat-anti-mouse antibody (dilution according to the manufacturer's indication) was added. A 30-minute incubation at 37°C was followed by several wash steps using PBS and thereafter the alkaline phosphatase detection reaction with alkaline phosphatase including developing solution (36 µM 5'-bromo-4-chloro-3-indolylphosphate, 400 µM nitroblue tetrazolium, 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) at room temperature until bands were visible.

It showed that the antibody ACC 2207 according to the invention recognizes specifically histidine fusion polypeptides but not polypeptides without histidine portion.

(b) **ELISA**

A 96-well plate was provided per well with 100 µl each, which included 20 ng and 8 ng, respectively, of the histidine fusion polypeptides and the controls of (a), respectively. After incubation at 4°C overnight, 3 short wash steps using PBS followed. Thereafter, the free binding sites of the polymeric carrier were blocked by one-hour incubation using 1 % BSA in PBS at 37°C. The antibody ACC 2207 according to the invention which was diluted in a ratio of 1:10 and 1:50, respectively, was incubated on the plate at 37°C for 1 hour. After 8 wash steps using PBS, the peroxidase-coupled goat anti-mouse antibody of (a) was added. A 30-minute incubation at 37°C was followed by 8 wash steps and thereafter the peroxidase detection reaction with developing solution (50 mM sodium acetate, 0.4 mM 3,3',5,5'-tetramethylbenzidine dihydrochloride, 4.4 mM H_2O_2) at room temperature until bands were visible.

It showed that the antibody ACC 2207 according to the invention recognizes specifically histidine fusion polypeptides but not a polypeptide without histidine portion.